Analysis of Chloramphenicol in Honey Using the SCIEX Triple Quad™ 3500 System

Santosh Kapil.G[1]; Chandrasekar[1].M; Aman Sharma[1]; Anoop Kumar[1]; Manoj G Pillai[1] & Jianru Stahl-Zeng[2]
[1]SCIEX, Udyog Vihar, Phase IV, Gurgaon - 122015, Haryana, India; [2]SCIEX, Darmstadt, Germany

Overview

The combination of liquid chromatography with tandem mass spectrometry (LC-MS/MS) allows identification and quantification of trace amount of chloramphenicol in complex food matrices due to the specificity and sensitivity associated with this technique. The SCIEX Triple Quad™ 3500 System enables labs performing antibiotic testing in foods to upgrade to LC-MS/MS and capitalize on its many benefits. The method development was performed according to the criteria established by the EC Commission Decision 657/2002[1]. This method described here demonstrates the ability of SCIEX Triple Quad™ 3500 to carry out quantitation and the confirmation of the chloramphenicol in honey at the required minimum required performance limit (0.3ng/ml).

Figure 1. SCIEX Triple Quad™ 3500

Introduction

Chloramphenicol has a wide spectrum of antimicrobial activity. It is effective against Gram-positive and Gram-negative cocci and bacilli (including anaerobes), Rickettsia, Mycoplasma, Chlamydia, among others. It is widely used as a human antibiotic and also as a veterinary drug. A triple quadrupole based method for the quantitation of Chloramphenicol in honey was developed using selective Multiple Reaction Monitoring (MRM). The ratio of quantifier and qualifier transition was used for compound identification. Sensitivity of detection met existing regulatory requirements, such as Codex Alimentarius' minimum required performance limit was 0.3ng/ml. The method was successfully applied to the analysis of store bought honey samples.

The SCIEX Triple Quad™ 3500 system takes the best features of the API 3200™ system and enhances them with modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.
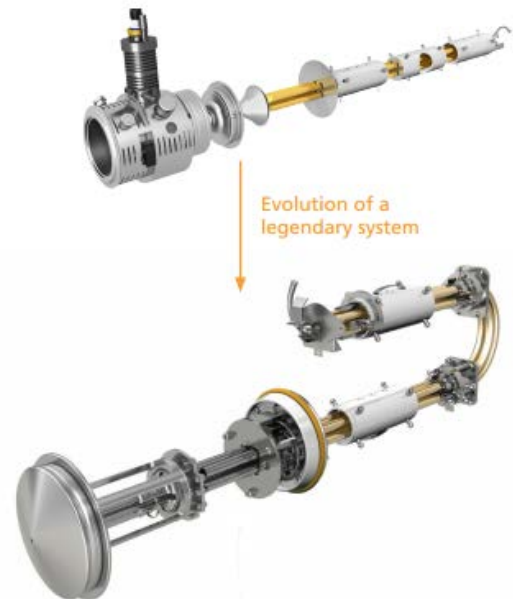

Evolution of a legendary system

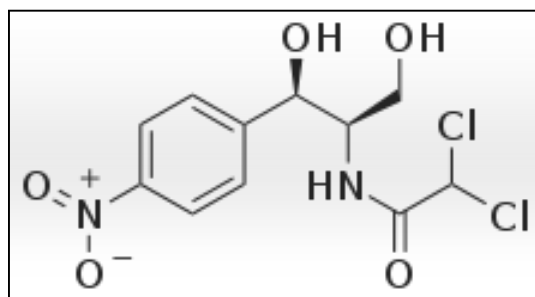Figure 2. Re-engineered quadrupole to maximize efficiency

Figure 3. Structure of Chloramphenicol $C_{11}H_{12}Cl_2N_2O_5$ 322.0123 g/mol

Unique Features

1. A sensitive, specific, rugged and reproducible LC-MS/MS method was developed for Chloramphenicol using simple Extraction technique for the sample preparation.

2. Accuracy and Precision for Chloramphenicol in honey samples found to be between 85-115%

3. Reproducibility of results for Chloramphenicol in terms of % CV in honey samples is less than 5%

4. Average recovery of Chloramphenicol in honey using the developed extraction method is more than 84%

Materials and Methods

Chemicals
Standard Chloramphenicol (with chemical purity ≥ 99%) was purchased from Clearsynth. All other chemicals used were of LC-MS grade.

Honey samples
Honey samples were procured from the local market of Delhi & Gurgaon in India and were kept at 2 - 8 °C until end of analysis.

Sample Preparation

1. Weighed 1 gm honey added 5 ml water and vortex well for 2 min.
2. Add 10 ml of Acetonitrile and 1gm of sodium chloride. Mix well.
3. Centrifuge for 5 min at 4000 rpm. Transfer the acetonitrile layer and evaporate under nitrogen steam.
4. Reconstitute with 1 ml diluent (water: acetonitrile 90:10), and used for LC-MS/MS analysis.

LC Conditions

LC separation was performed on a Shimadzu instrument using Synergy Fusion RP 18e (50 X 2.6) mm 2.5µ and a fast gradient of water (Mobile Phase A) and acetonitrile (Mobile Phase B) from 85% aqueous to 85% organic in 5 minutes at a flow rate of 0.4ml/min and injection volume of 20µl is used to obtain a good peak shape.

Time (min)	Mobile phase A%	Mobile phase B%
0.01	85	15
0.30	85	15
0.50	75	25
1.00	70	30
1.50	15	85
3.00	15	85
4.00	85	15
5.00	Controller	Stop

Table 1. Mobile Phase Gradient

MS/MS Conditions

The SCIEX Triple Quad™ 3500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe negative ionization mode at 2800 ion spray voltage, with Declustering potential(DP) -85V and Collision Energy(CE) -15V and -23V respectively. Two selective MRM transitions were monitored and ion ratio is calculated automatically by software for compound identification. Analyst® 1.6.2 Software was used for method development and data acquisition. LC-MS/MS data was processed using the MultiQuant™ Software version 3.0.1

Compound	Precursor ion	Product ion Quantifier	Product ion Qualifier
Chloramphenicol	320.8	151.8	256.8

Table 2. Selected MRM transitions
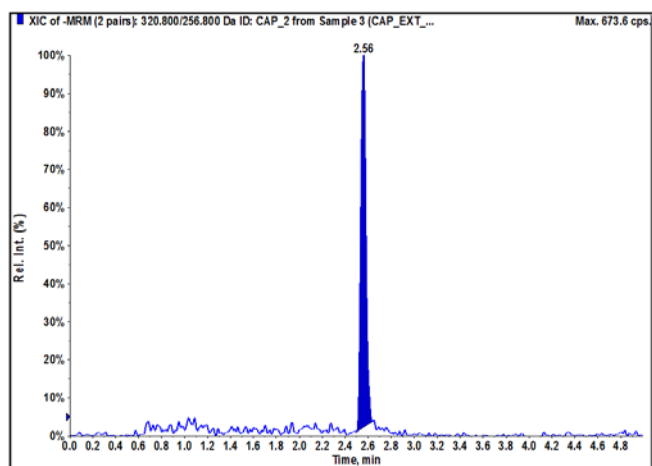
Results and Discussions

Figure 4. Representative Chromatogram of Chloramphenicol (0.1ng/ml) in Honey Matrix.

The calibration curves were made with standard levels ranging from 0.1ng/ml to 5.0ng/ml spiked concentration; 0.1ng/ml is set as the lowest linearity calibration point with the regression co-efficient (r): 0.99 by using weighing factor 1/X2.
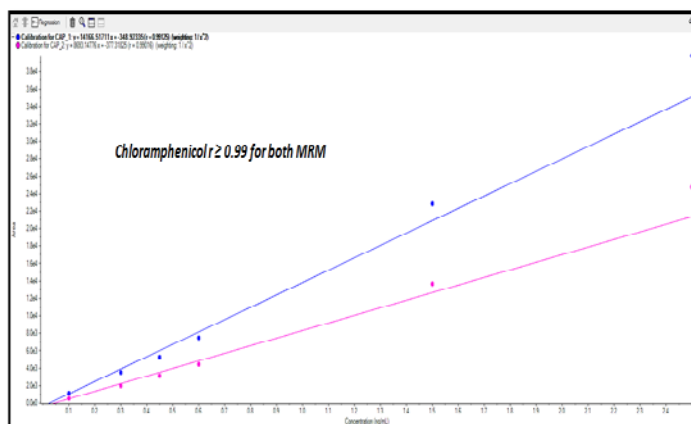

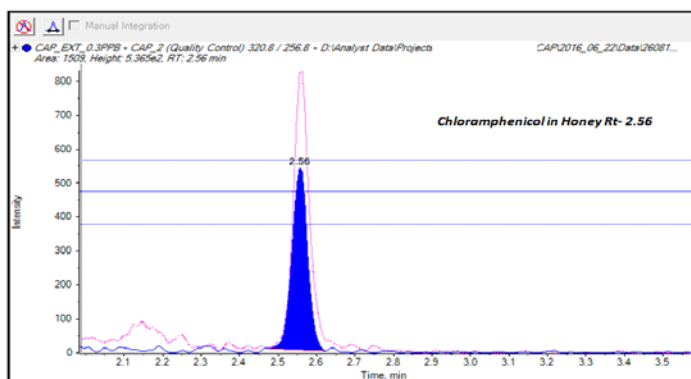**Figure 5. Linear range of the detection of Chloramphenicol from 0.1 to 5.0ng/mL (r = 0.99)**

Figure 6. MRM Ratio of Chloramphenicol at Rt- 2.56 was ≤ 1.0

Sample Name	Sample Type	Component Name	Area	Actual Concentration	Calculated Concentration	Accuracy	Retention Time	Used	MRM Ratio
CAP_EXT_BLK	Blank	CAP_2	N/A	N/A	N/A	N/A	N/A	☑	0.000
CAP_EXT_0.1PPB	Standard	CAP_2	544	0.10	0.1	106.04	2.55	☑	0.474
CAP_EXT_0.3PPB	Standard	CAP_2	1975	0.30	0.3	90.21	2.56	☑	0.563
CAP_EXT_0.45PPB	Standard	CAP_2	3094	0.45	0.4	88.73	2.55	☑	0.584
CAP_EXT_0.6PPB	Standard	CAP_2	4394	0.60	0.5	91.48	2.55	☑	0.586
CAP_EXT_1PPB	Standard	CAP_2	13696	1.50	1.6	107.93	2.55	☑	0.599
CAP_EXT_5PPB	Standard	CAP_2	24750	2.50	2.9	115.62	2.55	☑	0.622

Table 3. Accuracy data obtained for Chloramphenicol in the Honey matrix

Recovery was assessed by performing tests in honey samples in replicates (n=6) at MRPL concentration respectively. The Recovery of Chloramphenicol was ≥ 84% at RT of 2.56 minutes with minimum background noise in 5.0 minutes chromatographic run.

Replicates (n=6)	Chloramphenicol MRPL
1	0.300
2	0.250
3	0.250
4	0.250
5	0.270
6	0.260
Average Conc. (ng/mL)	0.263
Original Conc. (ng/mL)	0.300
% Recovery	87.78

Table 4. Recovery of Chloramphenicol in the Honey matrix at MRPL concentration level

Summary

The method and data presented here showcase the fast and accurate solution for the quantitation and identification of Chloramphenicol in honey samples by LC-MS/MS which meets the regulatory requirements. The SCIEX Triple Quad™ 3500 system provide excellent sensitivity and selectivity, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period. Automatic MRM ratio calculation in MultiQuant™ Software can be used for confirmation in compound identification.

References

1. ECFA, Who Food Additives Series, 53, 1–40, (2014)
2. European Commission Decision 2003/181/EC for setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin
3. FSSAI manual of methods of analysis of food.
4. A. Schreiber: 'MultiQuant™ Software Version 3.0 - Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples' Application Note AB SCIEX (2013) #8160213-01
5. Letícia R. Guidi, Luiza H. M. Silva, Christian Fernandes, Nicki J. Engeseth and Maria Beatriz A. Gloria; LC-MS/MS determination of chloramphenicol in food of animal origin in Brazil. Scientia Chromatographica 2015; 7(4): 287-295
6. EC European Commission, Commission Decision 2002/657/EC of 12 August 2002. Off. J Eur. Communities, L221, 8–36 (2002)

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices